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EXAMINER	
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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/620,433	LUDLOW ET AL.	
	Examiner	Art Unit	
	Anoop Singh	1632	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *06 July 2007*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-28 and 88-100* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-28, 88-100* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Applicant's amendment filed on July 6, 2007 has been received and entered. Claims 92 and 93 have been amended.

Claims 1-28 and 88-100 are pending in the instant application.

Election/Restrictions

Applicant's election with traverse of group I, claims 1-28, in the reply filed on April 14, 2006 was acknowledged. The traversal was on the ground(s) that the office has not demonstrated that it would be a burden to examine claims 1-87 together. This was not found persuasive because the office has demonstrated that the various claims fall into patentably distinct groups as supported by their different classifications. Further, each of the groups would require a unique search and require a different consideration of the relevant art within the scope of the invention as stated in previous office action dated 7/13/2006.

Claims 1-28 and 88-100 are under consideration.

Withdrawn-Objection-Specification

The objection to the disclosure is withdrawn in view of amendments to the specification. It is noted that applicant's have removed hyperlink and/or other form of browser-executable code from the specification.

Withdrawn-Claim Rejections - 35 USC § 112

Claims 92-93 rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement is withdrawn in view of amendments to the claims.

Maintained-Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (e) the invention was described in (1) an application for patent, published under section 122(b), by another filed in the United States before the invention by the applicant for patent or (2) a patent granted on an application for patent by another filed in the United States before the invention by the applicant for patent, except that an international application filed under the treaty defined in section 351(a) shall have the effects for purposes of this subsection of an application filed in the United States only if the international application designated the United States and was published under Article 21(2) of such treaty in the English language.

Claims 1-7, 9-13, 15-21, 26, 27 remain provisionally rejected under 35 U.S.C. 102(e) as being anticipated by copending Application No. 09/764,359 (US patent application no: 2002/0039786; art of record) which has a common inventor with the instant application. Based upon the earlier effective U.S. filing date of the copending application, it would constitute prior art under 35 U.S.C. 102(e), if published under 35 U.S.C. 122(b) or patented.

The cited art teaches methods of isolating liver progenitor cells comprising methods of fractionation by density centrifugation in particular the use of percoll gradients for separation of cell populations from the liver, in particular for the isolation of liver stem cells from primates such as humans.

The applied reference has a common inventor with the instant application. Based upon the earlier effective U.S. filing date of the reference, it constitutes prior art under 35 U.S.C. 102(e). This rejection under 35 U.S.C. 102(e) might be overcome either by a showing under 37 CFR 1.132 that any invention disclosed but not claimed in the reference was derived from the inventor of this application and is

thus not the invention "by another," or by an appropriate showing under 37 CFR 1.131.

Maintained-Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claims 1-2, 6 remain rejected under 35 U.S.C. 102(b) as being anticipated by Tateno et al (EP 682106, dated 11/15/1995).

Tateno et al teach a method to obtain liver parenchymal cell having clonal growth ability that are considered to contain hepatic progenitor cells (see abstract and page 3, line 4-5). It is noted that method disclosed by Tateno et al embrace isolating hepatic cells from liver of adult mammal by the collagenase perfusion and percoll centrifugation (see table 1, page 7). Tateno et al describe presence of at least two fractions upon centrifugation. It is noted that Tateno et al also disclose isolating cells from the light fraction after centrifugation that is then cultured in a medium containing FBS and ascorbic acid (see table 1 and claim 6 and 7). It is emphasized that instant method claim do not exclude other method of isolating hepatic cells such as standard percoll based isolation method. Accordingly, method steps taught Tateno et al recite same as one in the instant claims. Accordingly, claims1-2, 6 are anticipated by Tateno et al.

Response to Arguments

Applicants' arguments filed July 6, 2007 have been fully considered but they are not fully persuasive. Applicants in their argument on page 11, states that the claimed invention is a method of obtaining a population of cells enriched in human liver cells comprising, in part, "adjusting the density of the medium in which the cells are suspended whereby *at least two bands of cells separated by a density barrier are* obtained upon centrifugation." Applicants assert that percoll density gradient,

known at the time of invention, did not yield, upon centrifugation, *at least two*

bands of cells separated by a density barrier. Applicants also argue that a Percoll density gradient did not produce any bands of cells and percoll density gradient as taught in the art, including the copending application, separates cells in three fractions: a pellet at the bottom of the density gradient, afloat atop the density gradient, and interspersed *throughout* the density gradient.

In response, it is noted that the evidence of record present in US patent application no: 2002/0039786 teach a method to of isolating liver progenitor cells comprising methods of fractionation by density centrifugation in particular the use of percoll gradients for separation of cell populations from the liver, in particular for the isolation of liver stem cells from primates. It is known in the art that the density of stock isotonic percoll solution ('100% Percoll') is 1.123 g/ml and further dilutions to lower densities with 1XPBS gives a linear correlation between concentration and density. Furthermore, isotonic percoll is mixed with the sample and the gradient is formed in situ by centrifugation. Cells and particles band at their buoyant density during the centrifugation. With respect to applicant's argument that cited reference fail to teach adjusting the density of the medium, it is noted that the M-W dictionary defines the term "adjusting" to " bring to a more satisfactory state". In the instant case, recitation of adjusting the density of the medium is not a physical limitation rather it could be broadly interpreted as changing pH or condition or volume of the medium to bring to the cells to more satisfactory state in which cells are suspended to separate in at least two bands of cells. It is emphasized that a more specific physical limitation or condition recited in the claims that distinguish two bands would obviate the basis of this rejection.

With respect to applicant's argument that cited reference fail to disclose any band, it is noted that applicants agree that cited references teaches at least two fraction of cells one at the top while other at the bottom. MW dictionary defines band as "something that confines or constricts while allowing a degree of movement, narrow strip" (http://m-w.com/dictionary/). It is emphasized that cited references

meets the claim limitation as it teaches conditions wherein two distinct population of cells aggregate upon centrifugation to form two distinct layer of cells. The formation of at least two fractions one afloat atop the density gradient and one at the bottom of the Percoll that are separated by density barrier formed after centrifugation. The art further teaches collecting band of lower density meeting the claim limitation. Furthermore, contrary to applicant's argument the claimed method step does not exclude presence of other interspersed cells throughout the density gradient. Therefore, rejection to instant claim is maintained for the reasons of record.

Tateno et al

Applicant's arguments filed July 6, 2007 have been fully considered but they are not fully persuasive. Applicants in their argument asserts that standard Percoll-based isolation method does not have a density such that at least two bands of cells separated by a density barrier are obtained upon centrifugation. Applicants argue that instant claims do not form two discrete bands as claimed. Applicants assert that the "light fraction" is the population of cells that float atop the density gradient upon centrifugation and the "heavy fraction" is presumably the pellet of cells. The presently claimed invention, requires the generation and isolation of cells from the lower density band of two *bands* of cells separated by a density barrier.

With respect to applicants argument of adjusting the density, Examiner has already described that "adjusting of the medium" is not a physical limitation rather it could be broadly interpreted as changing pH, condition or volume of the medium to bring to the cells to more satisfactory state in which cells are suspended to separate at least two bands of cells. It is generally known in the art that the density of stock isotonic percoll solution ('100% Percoll') is 1.123 g/ml and further dilutions to lower densities with 1XPBS gives a linear correlation between concentration and density. It is further known that isotonic percoll is mixed with the sample and the

gradient is formed in situ by centrifugation. Cells and particles band at their buoyant density during the centrifugation. It is also known that sample could be layered at different place for separation of population of cells. During centrifugation in a swinging-out rotor with the tube in a vertical position, at low speed, the cells start to move in the direction of gravity. Simultaneously a gradient is formed by the sedimentation of Percoll particles. Cellular particles separate according to size or density depending on centrifugation time and g-force used. Thus, Tateno et al disclose isolating cells from the light fraction afloat atop the density gradient that would be cells from lower density as compared to cells collected at bottom, which would be of higher density (see table 1 and claim 6 and 7). Therefore, in absence of any specific physical limitation of adjusting the density of the medium (emphasis added) to separate band of different density Tateno et al meets the limitation of instantly claimed method.

Maintained- Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 1-10, 12-17, 26 remain rejected under 35 U.S.C. 103(a) as being unpatentable over Tateno et al (EP 0682 106 A2, dated 04/11/1995), Singh et al Acta Physiol Scand 117(4): 497-505, April 1983, art of record) and Naughton et al (US Patent no. 5785964, dated 7/28/1998).

Tateno et al teach a method to obtain liver parenchymal cell having clonal growth ability that are considered to contain hepatic progenitor cells (see abstract and page 3, line 4-5). It is noted that method disclosed by Tateno et al embrace isolating hepatic cells from liver of adult mammal by the collagenase perfusion and

percoll centrifugation (see table 1, page 7). Tateno et al describe presence of at least two fractions upon centrifugation. It is noted that Tateno et al also disclose isolating cells from the light fraction after centrifugation that is then cultured in a medium containing FBS and ascorbic acid (see table 1 and claim 6 and 7). Although, Tateno et al teach a method to isolated hepatic cell including progenitor cells and generally embraced the idea that liver parenchymal cells have clonal growth and contains hepatic progenitor cells, which could be selected or enriched with specific markers, however, Tateno et al differed from claimed invention by not explicitly disclosing isolation of different nonparenchymal cells.

Prior to instant invention, Singh et al disclose a method to separates viable rat liver parenchymal cells from other cell populations present in crude suspensions of liver cells. Singh et al disclose fractionation of cells by centrifugation in a self-generated Percoll gradient (see abstract). It is noted that Singh et al disclosed that method could separate Kupffer cells from other sinusoidal cells, resulting in a separate peak of peroxidase negative non-parenchymal cells. However, Singh et al do not explicitly disclose use of mechanical dissociation of cells.

Naughton et al teach a method to culture a variety of different cells and tissues in vitro for prolonged periods (see abstract). Naughton et al teach that the tissue or organ could be disaggregated mechanically and/or treated with digestive enzymes and/or chelating agents to weaken the connections between neighboring cells enabling to disperse the tissue into a suspension of individual cells. Naughton et al also disclose that enzymatic dissociation could be accomplished by mincing the tissue and treating the minced tissue with any of a number of digestive enzymes including collagenase, elastase and/or hyaluronidase, DNase, pronase, dispase. Naughton et al contemplate various methods for mechanical disruption including using grinders, blenders, sieves, homogenizers, and pressure cells. Naughton et al also teach method comprising exposing the hepatic portal vein by dissection and then perfusing with collagenase solution separating the outer parenchyma and mincing the inner parenchyma in Hanks balanced salt solution (HBSS). Naughton et al disclose centrifuging the cells through a Percoll gradient and separating liver parenchyma cells. Although, Naughton et al generally embraced the idea of separating nonparenchymal cell from liver using percoll centrifugation method, he did not contemplate using method for isolating hepatic progenitor cells.

Accordingly, in view of the teachings of Tateno, Singh and Naughton et al, it would have been obvious for one of ordinary skill in the art, at the time the claimed invention was made, to modify the method of dissociating cell from liver as disclosed by Tateno by mechanical dissociation or using other protease such as elastase with a reasonable expectation of success. One of ordinary skill in the art would have been sufficiently motivated to make such a modification since Naughton et al had successfully taught isolation of cells using mechanical dissociation and also emphasized the use of different protease for enzymatic. The artisan would be further motivated to use different method of dissociation in order to maximize the

isolation of cell, particularly since both Tateno and Naughton et al sought to isolate hepatic cells using percoll gradient centrifugation. Therefore, given that other mechanical or enzymatic dissociation method were available for isolation of different hepatic cells as per the teachings of Naughton et al, it would have obvious for an artisan of ordinary skill to use Percoll gradient centrifugation by digesting liver with different protease or by mechanical dissociation as disclosed in the instant application.

One who would practiced the invention would have had reasonable expectation of success because Tateno, Singh and Naughton et al had already described the method to isolate hepatic cells using Percoll density gradient centrifugation method. Thus, it would have only required routine experimentation to modify the method disclosed by Tateno to include other method to digest liver to isolate hepatic cells including progenitor cells as disclosed by instant invention.

Thus, the claimed invention, as a whole, is clearly *prima facie* obvious in the absence of evidence to the contrary.

Claims 1-6, 8, 11-17, 22-28, 88-100 remain rejected under 35 U.S.C. 103(a) as being unpatentable over Tateno et al (EP 0682 106 A2, dated 04/11/1995), Brill et al (Proc Soc Exp Biol Med. 1993; 204(3): 261-9), Cassiman et al (Am J Pathol. 1999; 155(6): 1831-9) and Graham (Scientific World J 2:1347-50, May 2002).

Tateno et al teach a method to obtain liver parenchymal cell having clonal growth ability that are considered to contain hepatic progenitor cells (see abstract and page 3, line 4-5). It is noted that method disclosed by Tateno et al embrace isolating hepatic cells from liver of adult mammal by the collagenase perfusion and percoll centrifugation (see table 1, page 7). Tateno et al describe presence of at least two fractions upon centrifugation. It is noted that Tateno et al also disclose isolating cells from the light fraction after centrifugation that is then cultured in a medium containing FBS and ascorbic acid (see table 1 and claim 6 and 7). Although, Tateno et al teach a method to isolated hepatic cell including progenitor cells and generally embraced the idea that liver parenchymal cells have clonal growth and contains hepatic progenitor cells, which could be selected or enriched with specific markers. However, Tateno et al differed from claimed invention by not using Optiprep (iodixanol) for isolation and enrichment of smaller heaptocytes and cells of 7-12 microns.

Brill et al teach a method for identifying and isolating antigenically related cell populations present in normal tissues using monoclonal antibodies to oval cell antigens and fluorescence-activated cell sorting. It is noted that Brill et al disclose three cellular subpopulations that could be isolated including (i) committed progenitors to heaptocytes; (ii) committed progenitors to bile ducts; or (iii) a mixed

population of hemopoietic cells that contained a small percentage of hepatic blasts that are possibly pluripotent. Brill et al also teach that the hepatic blasts are small (7-10 microns) cells that differentiate into cells with recognizable parenchymal cell fates (see abstract). Thus, any marker associated with progenitor cells of 7-10 micron is implicit in the teaching of Brill et al. It is noted that although Brill et al provided adequate guidance of presence of distinct population of hepatic cell including hepatic progenitor, he did not teach method to isolate small size cell using Optiprep (iodixanol) based gradient centrifugation method for isolation of cells.

Cassiman et al teach a method to isolate hepatic stellate cells of low density by collagenase/pronase digestion followed by density gradient centrifugation with iodixanol (Optiprep) It is noted that Cassiman et al disclose treating liver tissue with collagenase type IV (0.05% w/v) and digestion with Pronase E. Cassiman et al also teach isolation of cells at densities <1.053 (9% Optiprep) using the method incorporated by the reference of Alpini describing advances in isolation of liver cells. Cassiman et al differed from claimed invention by not disclosing isolation of hepatic cell includes progenitor cells.

Graham et al teach that majority of parenchymal cells from mammalian liver cells can be removed by very low speed centrifugation (50 g) but a simple low-density barrier (1.096 g/ml) is required to remove the remaining parenchymal cells from the supernatant which contains all of the lower density nonparenchymal cells. Graham et al disclose flotation through a low-density iodixanol barrier could provide a satisfactory enrichment of the least dense nonparenchymal cell and the stellate cells. It is noted that Graham disclose that low density nonparenchymal cell could be isolate or enriched using low-density iodixanol (optiprep) barrier, however, Graham et al do not teach the method steps to isolate cells.

Accordingly, in view of the teachings of Tateno, Brill, Cassiman and Graham, it would have been obvious for one of ordinary skill in the art, at the time the claimed invention was made, to modify the method of obtaining hepatic cells by replacing the percoll based cell separation medium with a iodixanol (optiprep) barrier based density gradient with a reasonable expectation of success. One of ordinary skill in the art would have been sufficiently motivated to make such a modification, as Brill had already disclosed the presence of mixed cellular subpopulations that could be isolated separately including committed progenitors to hepatocytes; committed progenitors to bile ducts; or a mixed population of hemopoietic cells. The artisan would be further motivated to use flotation of cells through a low-density iodixanol barrier to provide satisfactory enrichment of the least dense nonparenchymal cell and the stellate cells as taught by Graham, particularly since the hepatic blasts are only 7-10 microns (supra) and both Tateno and Graham et al sought to isolate hepatic cells. Thus, any marker such as one CD133 or EP-CAM associated with cells of 7-10 micron size derived from liver is implicit in the teaching. Although Tateno et al did not use low-density iodixanol barrier, he generally embraced potential of density based separation method for the

isolation of hepatic cell including progenitor cells. In addition, Tateno et al and Brill provided motivation of using other separation method by suggesting presence of hepatic progenitor cell of smaller size. Therefore, given that low-density iodixanol barrier based gradient centrifugation method were available for isolation of different hepatic cells as per the teachings of Cassiman et al and Graham et al, it would have obvious for an artisan of ordinary skill to use iodixanol based density gradient centrifugation method to isolate hepatic cell including progenitor cells as disclosed in the instant application.

One who would practiced the invention would have had reasonable expectation of success because Tateno, Cassiman and Graham had already described the method to isolate hepatic cells using density gradient centrifugation method. Tateno and Brill, had already described the presence of low density nonparenchymal cell, while Graham et al suggested that low density low density nonparenchymal cell could be enriched using iodixanol based method. Thus, it would have only required routine experimentation to modify the method disclosed by Tateno to include with a iodixanol (optiprep) based method to isolate hepatic cells including progenitor cells as disclosed by instant invention.

The limitation of claims 13-17 and 90-91 and 98-100 are included in the instant rejection since these buffer comprising RPMI-1640 medium with 10% human or bovine serum, or filtering step, centrifugation speed and machine, collection bags as required by the claims are obvious variations of the medium, filtration speed, machine and collection bags disclosed by cited arts. It is emphasized that in absence of any unexpected result one of ordinary skill would have been sufficiently aware of the different analogous medium in presence or absence of phenol red or centrifugation machine and relative g force depending upon the rotor radius.

Thus, the claimed invention, as a whole, is clearly *prima facie* obvious in the absence of evidence to the contrary.

Claims 1-17, 22-28, 88-100 remain rejected under 35 U.S.C. 103(a) as being unpatentable over Tateno et al (EP 0682 106 A2, dated 04/11/1995), Brill et al (Proc Soc Exp Biol Med. 1993; 204(3): 261-9), Cassiman et al (Am J Pathol. 1999; 155(6): 1831-9), Graham (Scientific World J 2:1347-50, May 2002) and Naughton et al (US Patent no. 5785964, dated 7/28/1998).

The combined teaching Tateno et al, Brill et al, Cassiman et al and Graham have been discussed above and relied in same manner here. However, none of the references teaches mechanical dissociation or use of different protease.

Prior to filing of this application, Naughton et al teach that cells have been routinely harvested from tissue or organ by mechanically dissociation and/or

treatment with digestive enzymes and/or chelating agents to weaken the connections between neighboring cells enabling to disperse the tissue into a suspension of individual cells. Naughton et al also disclose that enzymatic dissociation could be accomplished by mincing the tissue and treating the minced tissue with any of a number of digestive enzymes including elastase and/or pronase. However, Naughton et al do not specifically teach isolation of cells using iodixanol (optiprep) based method.

Accordingly, it would have been obvious and within the scope of one of ordinary skill in the art to modify the method of obtaining cells enriched in viable human hepatic cells taught by Tateno et al, Brill et al, Cassiman et al and Graham et al to by using other method to dissociate cells from the liver as disclosed by Naughton et al.

One of ordinary skill in the art would have been motivated to mice or mechanically dissociate or use other protease to obtain population of hepatic cell particularly since Naughton et al had already disclosed that these could be used in conjunction with collagenase in order to obtain cells derived from liver.

The limitation of claims 13-17 and 90-91 and 98-100 are included in the instant rejection since these buffer comprising RPMI-1640 medium with 10% human or bovine serum, or filtering step, centrifugation speed and machine, collection bags as required by the claims are obvious variations of the medium, filtration speed, machine and collection bags disclosed by cited arts. It is emphasized that in absence of any unexpected result an artisan of ordinary skill would have been sufficiently aware of the different analogous medium in presence or absence of phenol red or centrifugation machine and relative g force depending upon the rotor radius.

Therefore, the claimed invention would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention.

Claims 1-28, 88-100 are rejected under 35 U.S.C. 103(a) as being unpatentable over Tateno et al (EP 0682 106 A2, dated 04/11/1995), Brill et al (Proc Soc Exp Biol Med. 1993; 204(3): 261-9), Cassiman et al (Am J Pathol. 1999; 155(6): 1831-9), Graham (Scientific World J 2:1347-50, May 2002) and further in view of Dementrious et al (US patent no. 6,140123, dated 10/ 31/ 2000, effective filing date 10/7/1998).

The combined teaching Tateno et al, Brill et al, Cassiman et al and Graham have been discussed above and relied in same manner here. However, none of the references teaches that the cells that are subjected to cryopreservation.

Prior to filing of this application, Demetriou et al teach that cells have been routinely harvested and preserved in scientific research and development. It is also noted that Demetriou et al teach that cell could be re-used after thawing and placing in a cell culture medium. Demetriou et al also disclose storage medium for cryopreservation (col. 1-2) including cryopreservation buffer comprising serum and DMSO (See entire col. 6 and 7). However, Demetriou et al do not specifically teach cryopreservation of hepatic cells.

Accordingly, it would have been obvious and within the scope of one of ordinary skill in the art to subject the method of cultured cells taught by Tateno et al, Brill et al, Cassiman et al and Graham et al to cryopreserve as taught by Demetriou et al.

One of ordinary skill in the art would have been motivated to cryopreserve expanded hepatic cells including progenitor cells for future analysis or use as described by Demetriou et al.

Therefore, the claimed invention would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention.

Response to Arguments

Tateno, Singh and Naughton

Applicant's arguments filed July 6, 2007 have been fully considered but they are not fully persuasive. Applicants' in their argument assert that invention recites a step of adjusting the density of the medium in which the cells are suspended whereby at least two bands of cells separated by a density barrier. Applicants argue that each of the cited references use a standard Percoll gradient, which gradient is not adjusted such that *at least two bands* of cells separated by a density barrier. Hence, the cited references, alone or in combination, do not arrive at the claimed invention. The standard Percoll method can not arrive at the claimed invention (at least with respect to step (c) of claim 1), one or ordinary skill in the art simply could not have had a reasonable expectation of success in using the Percoll method to arrive at the claimed invention.

With respect to applicant's argument that cited method fails to teach adjusting the density of the medium, it is reiterated that term "adjusting" is broad and which is defined as " to bring to a more satisfactory state" (MW dictionary) is

not a physical limitation. Therefore in absence of any physical limitation a process of changing pH or condition or volume of the medium to bring to the cells to more satisfactory state in which cells are suspended to separate at least two population of cells separated by a density barrier would meet the claim limitation. As discussed in preceding section, it is generally known in the art that the density of stock isotonic percoll solution ('100% Percoll') is 1.123 g/ml and further dilutions to lower densities with 1XPBS gives a linear correlation between concentration and density. It is further known that isotonic percoll is mixed with the sample and the gradient is formed in situ by centrifugation. Cells and particles band at their buoyant density during the centrifugation. In the instant case, Tateno teaches isolating hepatic cells from liver of adult mammal by the collagenase perfusion and percoll centrifugation and presence of at least two fractions upon centrifugation. It is noted that Tateno et al also disclose isolating cells from the light fraction afloat a the top of the gradient which is separated by density gradient to the fraction of cells at the bottom. Singh et al disclose a method to separates viable rat liver parenchymal cells from other cell populations present in crude suspensions of liver cells by centrifugation in a self-generated Percoll gradient (see abstract). It is generally known to one of ordinary skill in the art that when self-forming gradients of Percoll are used, one preferably choose a density at the center of the tube that is in between the density of the particles to be separated. The length and volume of the tube, type of rotor and, desired shape of the gradient, volume of the tube, type of rotor, and density of the gradient determine the time and speed to be used for forming the gradient is optimized depending upon the cell to be separated. In fact, Singh et al cell disclose banding of different cells at different density (see page 499, col.2, para.2 and page 501, col. 1, para. 1). It is well established in case law that a reference must be considered not only for what it expressly teaches, but also for what it fairly suggests. In re Burkel, 201 USPQ 67 (CCPA 1979). Furthermore, in the determination of obviousness, the state of the art as well as the level of skill of those

in the art is important factors to be considered. The teaching of the cited references must be viewed in light of these factors. It is emphasized that a 103 rejection the references must be considered as a whole. For the purpose of combining references, those references need not explicitly suggest combining teachings, much less specific references. In re Nilssen, 7 USPQ2d 1500 (Fed. Cir. 1988). In the instant case, combination of references provided adequate guidance with respect to known techniques to make continuous self forming or discontinuous gradient that would have been obvious to one of ordinary skill in the art to combine with method of Tateno in order to improve the method to separating cells in two different distinct population of cell aggregate (band of cells) that are separated by density gradient with reasonable degree of predictability. Furthermore, in absence of any specific physical limitation of adjusting the density of the medium (emphasis added) to separate bands of different density the, rejection to instant claims are maintained for the reasons of record.

Tateno, Brill et al, Cassiman and Graham

Applicant's arguments filed July 6, 2007 have been fully considered but they are not fully persuasive. Applicants in their argument assert that Tateno and Brill teach the use of a standard Percoll gradient, which gradient, for the reasons mentioned, does not have the advantages of the present invention. In fact, Applicants submit respectfully that Tateno and Brill teach away from the present invention insofar as the standard Percoll method excludes hepatic stem/progenitor cells from the isolate. In addition, applicants assert that Cassiman and Graham recite the use of iodixanol gradients. However, both of these references do not use (and therefore disclose) iodixanol gradients for the isolation of hepatic progenitor cells. Applicants also agree with the Examiner "Graham et al do not teach the method steps to isolate cells." What is more, neither reference teaches "centrifuging

... to obtain at least one band enriched for viable cells" as in claim 94 or at least two bands as in claim 1. In fact, Graham teaches the collection of cells "at the interface between the GBSS [media] and the 11.5% iodixanol (see Fig. 1)." Page 1349, step no. 5 under "stellate cells." Graham also suggests that Cassiman's cells are collected in a similar manner. *See* item no. 1 under "notes," page 1349. Hence, these references alone or in combination cannot properly sustain *a prima facie* case for obviousness as none of the references, alone or in combination, teaches each of the limitations of the claimed invention.

In response, it is reiterated that the test for combining references is not what the individual references themselves suggest, but rather what the combination of disclosures taken as a whole would have suggested to one of ordinary skill in the art. In re McLaughlin, 443 F.2d 1392, 170 USPQ 209 (CCPA 1971). For the purpose of combining references, those references need not explicitly suggest combining teachings, much less specific references. In re Nilssen, 7 USPQ2d 1500 (Fed. Cir. 1988). The reference of Tateno et al teach a method to isolated hepatic cell including progenitor cells and generally embrace the idea that liver parenchymal cells have clonal growth and contains hepatic progenitor cells that is separated in two distinct population of cells separated by a density barrier, however, Tateno et al differed from claimed invention by not using iodixanol that form continuous density gradient for isolation and enrichment of smaller heaptocytes and 7-12 microns size hepatic progenitor cells. Graham and Cassiman et al provide adequate guidance with respect to availability and use of iodixanol for density gradient centrifugation to separate cell population. It is noted that contrary to applicant's argument Graham et al disclose that the density solutions can be made up by dilution of OptiPrep (iodixanol) and different hepatic cells could be separated by adding cells to the iodixanol and then centrifuging results in isolation of cells at the densities 1.067 which is at the interface of iodixanol and buffer (densities 1.067) . Examiner would agree that Graham did not explicitly disclose the method to enrich hepatic

progenitor cells but clearly disclose digesting liver tissue and then suspending the crude nonparenchymal cells in GBS buffer so that the final concentration of iodixanol is 17% (w/v) iodixanol solution (p = 1.096 g/ml) upon centrifugation of the tube forms layer of cells that are separated to obtain hepatic cells. Thus, given the fact that one of ordinary skill in the art is aware that various factor such as the length and volume of the tube, type of rotor and desired shape of the gradient, volume of the tube, type of rotor, and density of the gradient determine the time and speed to be used for forming the gradient is optimized depending upon the cell to be separated. It is reasonable to state that it would have been *prima facie* obvious to one of ordinary skill in the art to apply the technique of separating hepatic cells in gradient formed by percoll by using iodixanol to improve the separation technique particularly since it could be made by dilution of OptiPrep directly with Gey's Balanced Salt Solution (GBSS), while others such as Nyeodenz require dilution with GBSS without NaCI to keep the osmolality below 300 nm (see page 1348, para. 2). Given that Graham et al had already disclosed feasibility of using iodixanol for gradient centrifugation and to separate different fractions of cells and more specifically modifying iodixanol concentration resulted in different resulting densities (1.0677 or 1.053). It would be obvious to one of ordinary skill in the art to improve the method of Tateno by using other gradient barrier such as one disclosed by Graham and Cassiman. It is noted that claim 94 does not require appearance of cells at the any specific density. In fact, limitation of claim 94 is completely taught alone by Graham and Cassiman. In summary, in absence of any physical limitation of adjusting the density as discussed in preceding section, and given that low-density iodixanol barrier based gradient centrifugation method were available for isolation of different hepatic cells as per the teachings of Cassiman et al and Graham et al, it would have obvious for an artisan of ordinary skill to use iodixanol based density gradient centrifugation method to isolate hepatic cell including

progenitor cells with routine optimization of volume and density of iodixanol as disclosed in the instant application

In response to Applicants argument that none of these five references, alone or in combination fails to teach collecting cells from a discrete band formed upon centrifugation, it is noted that cells appearing as light fraction at the top or cells or at the bottom upon centrifugation of continuous percoll gradient are considered as two population of cell aggregate which is separated by density gradient. As discussed before, the term adjusting.. is broad and is not a physical limitation. The reference of Graham and Cassiman teaches availability and extensive use iodixanol for gradient centrifugation to separate different fractions of cells and more specifically modifying iodixanol concentration resulted in different resulting densities (1.0677 or 1.053). It would prima facie obvious for one of ordinary skill in art to modify the concentration of iodixanol and centrifugation speed to separate cell of interest.

Maintained-Double Patenting

The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right to exclude" granted by a patent and to prevent possible harassment by multiple assignees. A nonstatutory obviousness-type double patenting rejection is appropriate where the conflicting claims are not identical, but at least one examined application claim is not patentably distinct from the reference claim(s) because the examined application claim is either anticipated by, or would have been obvious over, the reference claim(s). See, e.g., *In re Berg*, 140 F.3d 1428, 46 USPQ2d 1226 (Fed. Cir. 1998); *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); and *In re Thorington*, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) or 1.321(d) may be used to overcome an actual or provisional rejection based on a nonstatutory double patenting ground provided the conflicting application or patent

either is shown to be commonly owned with this application, or claims an invention made as a result of activities undertaken within the scope of a joint research agreement.

Effective January 1, 1994, a registered attorney or agent of record may sign a terminal disclaimer. A terminal disclaimer signed by the assignee must fully comply with 37 CFR 3.73(b).

Claims 1-28 remain provisionally rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 1, 3, 4, 6-9, 12-21, 23-34 of copending Application No. 09/764,359 (published as 2002/0039786 A1).

Although the conflicting claims are not identical, they are not patentably distinct from each other because each comprise methods of isolating liver progenitor cells comprising methods of fractionation by density centrifugation (compare claim 1 of the instant application with dependent claims 29 and 30 for example). It is noted that the set of claims do not recite each of the specific limitations in each case, however given the guidance of the two disclosures, the use of percoll gradients for separation of cell populations from the liver, in particular for the isolation of liver stem cells from primates such as humans, the two sets of claims would be obvious over each other.

Applicant's arguments with respect to claims 1-28 have been fully considered but they are not persuasive. It is emphasized that although the conflicting claims are not exactly the same, they are not patentably distinct from each other because both sets of claims encompass a method to separate cell population from the liver. As discussed before term adjusting is broad and is not a physical limitation and in absence of any such requirement instant claims are clearly anticipated by the copending application. Certain of the instant broader claims differ only with respect gradient medium that could be used for isolation of liver stem cell. A Terminal disclaimer or more specific amendment to differentiate instantly claimed gradient medium would obviate the basis of this rejection. This is a provisional obviousness-type double patenting rejection because the conflicting claims have not in fact been patented.

Conclusion

Gale et al. (J Endocrinol 92(2): 293-302, Feb 1982), teach a method for preparing leyding cells on a 0-60% linear density gradient of percoll. It is noted that leyding cells form a band corresponding to 35-50% percoll (density 1.050-1.07 g/ml), where as thick mass clumped together at a density corresponding to 25-20% percoll (density 1.035-1.045 g/ml).

No Claims allowed.

THIS ACTION IS MADE FINAL. Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Anoop Singh whose telephone number is (571) 272-3306. The examiner can normally be reached on 9:00AM-5:30PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Peter Paras can be reached on (571) 272- 4517. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

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